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Isolation of Chromosome 18-Specific Brain Transcripts as Positional Candidates for Bipolar Disorder

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Several studies have proposed the existence of susceptibility loci for bipolar disorder on chromosome 18. To identify possible candidate genes for this disease, we isolated brain-expressed transcripts by direct cDNA selection on chromosome 18-specific biotinylated cosmid clones. Longer cognate cDNA clones of the selected cDNAs were isolated from a normalized infant brain cDNA library. Physical mapping by PCR on a panel of somatic cell hybrids was conducted by the use of primers derived from partial sequences on either the 5′ or 3′ ends of the clones. In our initial analysis, 48 cDNA clones were found to be chromosome 18-specific, mapping to different subchromosomal regions. Sequence redundancy among these clones yielded 30 unique transcripts, five of which were represented in previously known genes. Further sequencing of the remaining 25 unique cDNA clones confirmed the absence of significant homology to known genes, indicating that these transcripts represented novel genes. Mapping with the use of a radiation hybrid panel positioned the brain cDNAs to within ≈100 to 1100 kb from reference sequence tag sites (STSs) and assembled them into six high resolution linkage groups. The majority of the transcripts were found to cluster to discrete locations on 18p and 18q, previously hypothesized as susceptibility regions for bipolar disorder, identifying them as positional candidate genes. Am. J. Med. Genet. 74:140–149, 1997. © 1997 Wiley-Liss, Inc.‡

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INTRODUCTION

Genome screening efforts by several groups, designed to identify regions linked to bipolar disorder, have revealed evidence for potential susceptibility loci on chromosome 18. Berrettini et al. [1994] reported evidence for a susceptibility locus in the pericentromeric region of the chromosome. In a subsequent study on an independent pedigree series, Stine et al. [1995] found support for the prior hypothesis on 18p [Berrettini et al., 1994]. In the same study, Stine et al. [1995] presented evidence for a possible additional linkage on 18q. Recently, Freimer et al. [1996] proposed a predisposing locus close to the telomere of 18q in Costa Rican kindreds. These reports suggest that the regions potentially implicated in bipolar disorder encompass a very large portion of chromosome 18.

In addition to bipolar disorder, more than 25 other diseases have been localized to chromosome 18, approximately 80% of which still await the discovery of the underlying defective gene [Overhauser et al., 1995; OMIM, 1996]. Since this chromosome has a genetic length estimated to be 150 cM [Cooperative Human Linkage Center (CHLC), 1994], which includes about 4.5% of the total length of the genome, it is expected to encode several thousand genes. Approximately 40

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genes have been mapped to this chromosome [Overhauser et al., 1995; Genome Database (GDB), 1996; OMIM, 1996]. Between 1993 and 1995, only 14 genes have been added to the list of chromosome 18 genes [Geurts van Kessel et al., 1994; Overhauser et al., 1995]. Therefore, a dense transcriptional map, which would be valuable in positional cloning of susceptibility genes, remains to be developed for chromosome 18.

Since narrowing down the region of linkage and identification of the mutated gene in a complex disease is difficult, we have focused our initial strategy on the detection of positional candidate genes for bipolar disorder. Here, we report the detection and mapping of 48 chromosome 18-specific, brain-expressed cDNAs, about half of which represent unique sequences encoded by novel genes.

METHODS

Chromosome 18-Specific Cosmid Clones Used for cDNA Selection

A human chromosome 18-specific cosmid library, LL18NCO2, was provided by the Human Genome Center at the Lawrence Livermore Laboratory. The source of the chromosomes was a human/hamster hybrid cell line X11-4A [Chang et al., 1993; Trask et al., 1991] retaining a single copy of chromosome 18 as its sole human material. The chromosomal DNA was partially digested with *Mbo*I, dephosphorylated, then ligated into the *Bam*HI site of the cosmid vector Lawrist 16 [Little, 1987; J. Garnes and P. de Jong, unpublished data]. The resulting arrayed library contained 145 96-well microtiter plates. A human genomic DNA probe hybridized to 84% of the clones in the library, 10% were positive with a rodent probe and the remaining 6% were nonrecombinants since they failed to hybridize with either probe. The chromosome 18 cosmid library represents 467 Mb [13,920 clones × 84% × 40 kb (assumed average size of cosmid insert)] in chromosomal coverage. Ten pools of the library were prepared by combining the contents of all wells from plates 1–10 (pool 1), 11–25 (pool 2), 26–40 (pool 3), etc. Cultures of the cosmid pools were grown in LB/kanamycin and the DNA isolated using the Qiagen plasmid kit (Qiagen). The DNA was biotinylated for 20 minutes using the Bio-Nick kit (GIBCO-BRL). The unincorporated nucleotides were excluded by ethanol precipitation.

Preparation of Primary cDNA

Total RNA was extracted from five regions of postmortem human brain (caudate, putamen, hippocampus, amygdala, frontal cortex) and from human placenta by acid-guanidine, phenol/chloroform method [Chomzynski and Sacchi, 1987]. Poly(A)$^+$ RNA was prepared using oligo(dT)-paramagnetic beads (Dynal), and double-stranded cDNA was synthesized with random priming using the Invitrogen Copy kit. The cDNA was subdivided into eight pools, each containing 1 μg of brain-derived cDNA and 0.8 μg of placental cDNA. A batch of total human brain poly(A)$^+$ RNA was purchased from Clontech (in order to represent regions of the brain not included above), and 4 μg of double-stranded cDNA was prepared as above. Each cDNA pool was ligated to an adaptor consisting of complementary oligonucleotides 1 and 2 [Lovett, 1994]. Since the brain tissues obtained were frozen following a postmortem delay, placental cDNA was added in the selection to retain transcripts (common to both brain and placenta) that might have been labile during this delay.

Direct cDNA Selection

Direct cDNA selection was performed using the magnetic bead capture technique described previously [Lovett et al., 1991; Lovett, 1994] to a Cot½ of 100, with some modifications. Briefly, repeats were blocked by mixing the starting cDNA pool with a mixture of low-molecular-weight Cot-1 DNA (2 μg per hybridization, GIBCO-BRL), high-molecular-weight Cot-1 DNA (20 ng per hybridization, GIBCO-BRL), and linearized cosmid vector DNA (30 ng per hybridization). The first round of selection was performed by hybridization of cDNA pools (1.8–2 μg each) and biotinylated cosmid pools (120 ng each). A second round of selection was conducted using 2 μg of amplified primary-selected cDNA and 120 ng of each biotinylated pool of cosmids. The PCR reactions for the primary- and secondary-selected cDNAs were performed using Expand Long Template PCR System (Boehringer Mannheim) with an initial denaturation at 94°C for 3 min, followed by 10 cycles of amplification at 94°C for 10 sec, 60°C for 30 sec, and 68°C for 3 min, and 25 cycles using the same denaturation and annealing conditions, and an autoextended elongation time of an additional 15 sec after every cycle.

Hybridization of High Density Filters of a Normalized Infant Brain cDNA Library

Approximately 40,000 clones from a normalized infant brain library constructed by Soares et al. [1994] were previously arrayed at the Lawrence Livermore Laboratory into 408 96-well microtiter plates. We rearrayed the library into 102 384-well microtiter plates and high-density filters were produced (service done by Research Genetics, Inc). One 22 × 22 cm filter contained 36,864 clones and the remaining 2,304 clones were spotted on another filter.

Each pool of amplified secondary-selected cDNA was labeled with [α-^{32}P]dCTP by random primer labeling (Boehringer Mannheim kit). One set of hybridizations of the high-density filters was done using a mixture of all the pools of labeled secondary selected cDNA, after a preblocking procedure using total human placental DNA, low-molecular-weight Cot-1, and linearized cosmid vector. Hybridization was done using 2×10^6 cpm of preblocked cDNA per ml of Rapid-hyb buffer (Amersham) at 65°C for 2 hr following a prehybridization of 1 hr. The final wash was in 0.1 × SSC, 0.1% SDS at 60°C. Using the same conditions, a replica filter was hybridized with 5×10^5 cpm/ml of ^{32}P-labeled human placental DNA.

Another set of hybridizations was performed using a mixture of two pools of secondary-selected cDNA. The hybridization pattern yielded by the secondary selected cDNAs was compared with that produced by human placental DNA. The clones corresponding to positive spots common to both filters were not picked due to the

possibility that the signals were from repeat hybridization. In addition, the hybridization patterns obtained with the cDNA subpools were compared with that produced using a combination of all secondary selected cDNA pools. All high and medium intensity clones were chosen, and clones that gave low intensity signals but were common to two or more filters were also picked (Fig. 1). The insert sizes were determined using the colony PCR method described previously [Yoshikawa et al., 1995].

Sequence Database Comparisons and Primer Design

The microtiter plate addresses of the positive clones chosen for further analysis were determined, and this allowed us to search the EST database (dbEST) [Boguski et al., 1993] permitting the retrieval of the IMAGE cDNA ID number and corresponding Genome Database (GDB) account number. Approximately 40% of the cDNA clones contained a short 3′ and/or 5′ end STSs that were deposited by the sequencing collaboration of Washington University and Merck & Co. For these available sequences, primers were designed using the program PRIMER v2.2 [Resnick and Stein, 1995], which had a T_m for primers set at between 52 and 55°C (Table I).

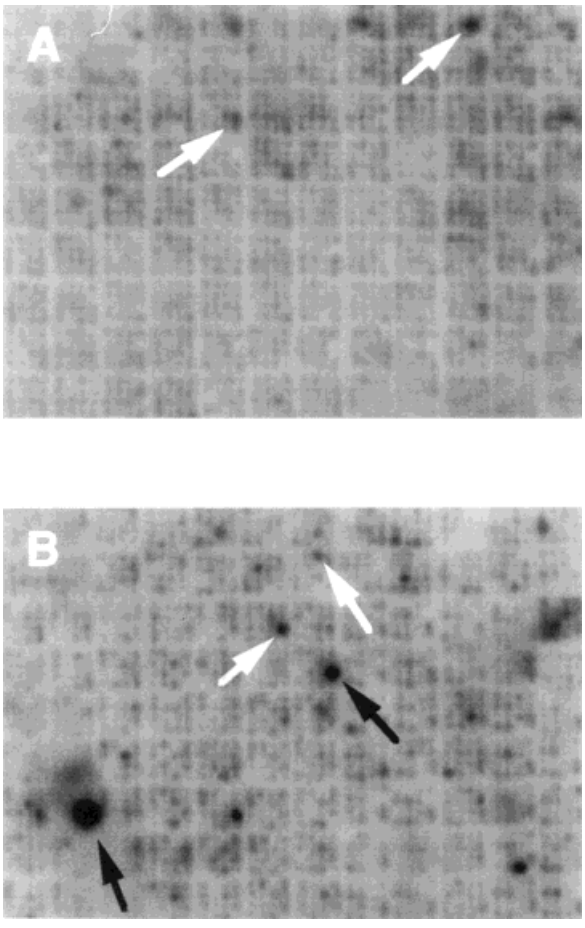

Fig. 1. Hybridization of high-density filters from fetal brain cDNA library. Two cycles of direct cDNA selection were performed using primary cDNA and chromosome 18 cosmid library. Amplified pools of secondary selected cDNA were labeled and used to probe high-density filters of a brain cDNA library [Soares et al., 1994]. (**A**) Shown is a region of a filter hybridized with a combination of all the pools of secondary-selected cDNA with medium (gray arrow) and low intensity signals (white arrow). (**B**) Shown is a region of another filter hybridized with a mixture of two pools of secondary-selected cDNA with high (black arrow), medium, and low intensity signals.

Mapping of cDNA Clones by PCR on Chromosome 18 Somatic Cell Hybrids

Genomic DNA was extracted from a panel of 20 somatic cell hybrids, one of which included the entire human chromosome 18 and the rest containing various segments of the chromosome [Overhauser et al., 1995]. A diagram of the hybrids used in this study is shown in Figure 2. Human genomic DNA and hamster genomic DNA were used as reference controls. Using this panel of chromosomal and genomic DNAs as template and primer pairs derived from each clone, mapping by PCR was conducted. If the initial primer pair failed to amplify, another pair was designed, or one of the primers in the original pair was modified.

PCR was performed using the Perkin Elmer Cetus GeneAmp System 9600. Amplification was done in a 20 µl reaction containing either 80 ng (somatic cell hybrid) or 30 ng (human or hamster genomic DNA) template DNA, 5 µM of each primer, 200 µM of each dNTP, and 0.75 unit of AmpliTaq (Perkin Elmer Cetus) in a standard PCR I buffer (Perkin Elmer Cetus). "Touchdown" PCR was done as follows: 30 sec at 94°C, 30 sec at $(T + 11 - n)$°C (T is listed in Table I and n is cycle number), 1 min at 72°C for the first 10 cycles, and 30 sec at 94°C, 30 sec at T°C, 1 min at 72°C in the subsequent 25 cycles. The PCR products were separated on 3% Nusieve:Seakem agarose gels.

Radiation Hybrid Mapping

The Stanford G3 radiation hybrid panel [Cox et al., 1990] (#RH01, available at Research Genetics, Inc.) was utilized to fine map the unique chromosome 18-specific brain cDNAs. This panel had a 500 kb resolution and an average of 26 kb/centiRay (cR), based on data available for chromosome 4 on 452 informative markers (http://shgc.stanford.edu/RHMap.html).

For radiation hybrid mapping, 40 ng of DNA from each of the 83 radiation hybrid cell lines was used as template, and PCR was performed with primers specific for a given cDNA clone (Table II). PCR was done in a 10 µl volume, and conditions were identical to those previously described for mapping with the chromosome 18 regional panel of somatic cell hybrids. Fifteen ng of human genomic DNA was used as positive control. The size of a PCR product, amplified from each radiation hybrid cell line, and a given pair of primers was determined by electrophoresis on a 3% Nusieve:Seakem agarose gel. For a given primer pair, the raw data indicating the presence or absence of an amplified product in each of the 83 radiation hybrid cell lines were submitted to the Stanford radiation hybrid e-mail server (http://shgc.stanford.edu/rhserver/intro.html). If linkage to reference markers was found, the mapping data

TABLE I. Primers Used for PCR Mapping

Clone number	Primer sequence		Product size (bp)
	Forward	Reverse	
1	5′-AGGAGTGGTGTACATTTCT-3′	5′-ACCTGCAACACATTAGAAAC-3′	134
2	GGTTTCTTCAAAATTTTATTAACAA	TCCTCCACTCATCTGTTTCT	175
3	CCTGACCTGATCAAGTTTA	GGTAAAGGAACAAGCTGC	125
4[a]	TGATCACACAGTCAGCACTGT	GGGCAGAAGTTTCCAATTACC	131
5	TATTGAGACCTAAGTCAGCATCC	GACAGAAAGCAGGTTAGAGGT	192
6	GAAACTTTACATCAGGTGTCTC	ATGGACTAGGAGTTTAAGC	283
7	GGAACAGTGTACACTTTCC	TATATAGCCTCGATGATGAGAG	185
8	CATGAGAGGAAGAGGTCTTTAT	GGGTTATGTCTTAGTCGAG	275
9	TCAGTAGAAACTCAAGCTGCTTC	CTCCCTCTCAGTGTGAGGCT	230
10	CCTGACCTGATCAAGTTTAA	TGTACACCACTCCTCATGT	179
11	CGACGACTCATACAACATATC	GGTTACAGCTGAAGTGTAT	177
12	TATTCAGGAACAGTGTACAC	TCGATGATGAGAGGGTTAC	174
13	GAACACTTATCTCCTTCTTCAG	TCCACTCCTTTCACCTCTTCT	243
14	AGACAAGAGCAAAACACAAC	CTCTTTGCAGTTCAGTCTA	169
15	AGGIGAACCATTTGACTGGTTT	GCTTGTGTGTGGCTGTCCTT	148
16	GGCTAAACTTACAGTATGTAAGGAG	CTGTAAGGACAGACTACTCA	152
17	CCAGGAGGITTCAGCGGT[d]	CGCAAAGCCATGAIAAACCG	115
18[b]	TCAGGAACAGTGTACACTTTC	TGTGGGCTTAATACCATGTCT	207
19	GGAATCTCTGTACTTGCT	GTGACACATTACAAAGCCA	154
20	TCAGTAGAAACTCAAGCTGC	CCTCTTCCTCTTAAAGTGT	101
21	TCACTTCAGAATCACTACTC	ACCCATCCTATATGAAAAGC	228
22	TACAAAAGAGGACAAAGCAC	GGTGCCTGTATATAAGTTGA	157
23	GGGATCATACTAAAGAGAAG	GGATAAACAGAGAGCTTGAT	193
24	CTACAGAATAGAATACATGGCG	GAGCTCTGAACTGTATTCAGA	224
25	GTCAGTTACTCTATTTGCTGTG	AACCTGTGCTGTAAAGTTCA	233
26	CTTAAGAGGAAGAGGCCAT	CTCTCCCTCTCAGTGTGAG	145
27	ACAATTAGGCATTGTTGATGG	CAGITCTTGCACATACAAGACA	112
28	ACCTTTGGCAAGGGGTATGA	TGTGAAGGCTGGGAAACACT	207
29	TCTCAGCTTACTCAACCT	GATGAGGTGGAACAATCAC	138
30	AACACTCAGCTCTGTAGAA	CGAGTCATCAATAGGACAA	212
31	GGTCTGTACAGTGTAATAAACC	CTACTGCAAAATGTGTCCTGTC	124
32	GAGCCAAGTGGAACTCTTGAA	GTCAGGAAAGAGGTTGTGAGC	156
33	ACACATATGTACACAGGAAC	TGTGTACAGCGAGTGAATTA	103
34	TTGTTCACACACAATCTAGG	ACTAGCATATCTGAATTCCCA	159
35	CTACAGAATAGAATACATGGCG	TTGAAACCAGACCCTGTAGT	166
36	CATTTAGTCCAGAGGCTCTT	TCCTCGAAGAGGTTGCAGC	161
37	CACATTAGCCAGTCTGATAAAG	AAGTTACACACAGTAGCTGA	107
38	CATTCAGCACACATAGAGTCTA	CCCTGTCCCTTGTATATGTA	189
39	AGTGTATCTACAACCTCAACTGTC	GTAAAGGCCCAATCAATGCACT	109
40	GCCAGATTCACAATTGATAG	CTGAAGGCACTTTATGTAC	139
41	CTGGAGCAGGTTAGATACACC	CTTCCCTCTTAACCTTTAGTGC	143
42	GTGTCTTGTATGTGCAAGAAC	GACTGGGTATCCTAGCTTAC	157
43[b]	TTAGTCAGACCCATTCAGTC	CCAGACTGCTTTATGTTAG	103
44[b]	GTGTCTTGTATGTGCAAGAAC	CCTAGCCTTACTGTTTAAC	146
45[a,c]	ACGATGCGATCCTGGAAG	CTGGCTTGAGTTTGTCTG	113
46[a,c]	CCTTTCTGTGTGAAGATCAC	AAGAAAGTCCCAAGGGTGGA	123
47[a]	GGAATGAGGGTTAGAGTCC	AGTGCTTCTGTAGCTCTT	114
48[a]	TGAGGGTGTGAACCACTCTG	GAATCCTGGTGTGCCCAAGT	137

[a] The 5′ portion of the insert was used. The remainder were from the 3′ portion.
[b,c] The T_m was [b]48°C or [c]60°C. The remainder were 52°C.
[d] An I in the primer sequence indicates the use of inosine for an unknown base.

transmitted from Stanford included a list of linked markers (STSs), lod scores, and distances in $cR_{(8000)}$. A lod score above 6 was used for assigning the unique clones to the Stanford framework map with a 95% confidence level.

RESULTS

cDNA Selection and Isolation of cDNA Clones from an Infant Brain Library

To isolate brain-expressed transcripts that map specifically to chromosome 18, we performed direct cDNA selection with pools of chromosome 18 biotinylated cosmid clones and primary cDNAs derived from human brain and placenta. After two cycles of selection, the secondary selected cDNA was PCR amplified, and this was found to have an average size of about 400 bp. Longer cognate cDNA clones were isolated by using labeled amplified pools of secondary selected cDNAs to probe high-density filters of an arrayed, normalized infant brain library [Soares et al., 1994] (Fig. 1). This strategy yielded a total of 174 positive cDNA clones. Analysis of the dbEST database revealed that less than

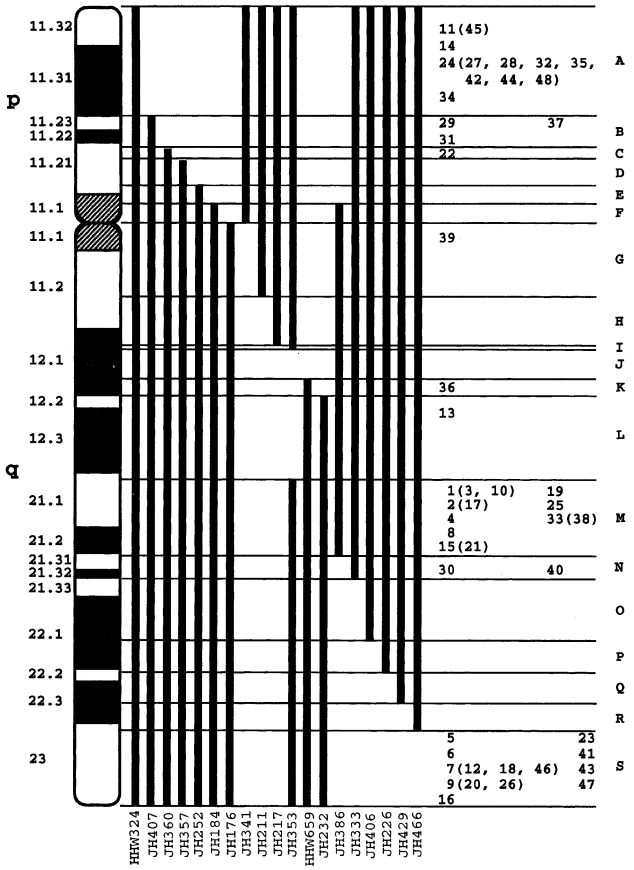

Fig. 2. Assignment of brain transcripts to chromosome 18 cytogenetic bins. cDNA selection yielded a total of 48 brain-expressed transcripts (numbered 1 to 48) that mapped specifically to the indicated regions of chromosome 18. Redundant transcripts are in parentheses next to the first member of each redundant group. The somatic cell hybrids that subdivide the chromosome into cytogenetic bins (represented by A to S, from pter to qter, right hand side) and the names of the cell lines (bottom) are indicated.

half of these clones had available sequences of a few hundred bp on the 3′ and/or the 5′ ends.

Initially, we focused our analysis on clones that had these partial sequences to facilitate rapid chromosomal localization by PCR. The availability of these sequences also permitted comparison with sequences in the databases for homology to known genes, and evaluation of possible redundancies between the selected transcripts.

Chromosomal Localization and Regional Mapping of Chromosome 18-Specific cDNAs

To determine the chromosomal location of the positive cDNA clones we designed PCR primers from the 3′ end sequence, whenever possible. Since the infant brain cDNA library was constructed by oligo(dT) priming and directional cloning this would most likely correspond to the 3′ untranslated region (UTR), which is usually unique and uninterrupted by introns [Sikela and Auffray, 1993]. Primers were developed to produce PCR products of less than 300 bp. Our analysis indicated that 83% of 3′ end-derived primer pairs and 74% of 5′ end-derived primer pairs amplified a PCR product with the expected size.

In the initial step of the clone-based physical mapping, a panel of template DNAs was used for PCR amplification. These included human placental DNA, somatic cell hybrid DNAs for the entire human chromosome 18 (HHW 324, Fig. 2) as well as segments (JH 353 and JH357, Fig. 2) of human chromosome 18, and hamster DNA. In addition, a number of somatic cell hybrid DNA isolates derived from other chromosomes were used as negative controls. After establishing that the cDNA was of human origin and was specifically localized to chromosome 18, mapping into subchromosomal regions was performed by PCR on a series of DNAs derived from somatic cell hybrids that subdivide the chromosome into cytogenetic bins (Fig. 2).

We found that the use of primers derived from 48 cDNA clones successfully amplified unique bands of the expected size, specifically on chromosome 18 somatic cell hybrid DNA (Table I). Further analysis using the same primer pairs (Table I) revealed that each of these 48 clones mapped to a specific chromosome 18 cytogenetic bin (Table II and Fig. 2), therefore, confirming our initial data on the chromosomal assignment. The remaining clones mapped either ambiguously or elsewhere in the genome. Interestingly, most of 48 brain transcripts appeared to cluster within discrete cytogenetic regions on chromosome 18: bins A and B, in the short arm and bins M and S, in the long arm (Table II and Fig. 2).

Sequence Homology Comparisons to Identify Unique Chromosome 18-Specific Transcripts

To determine the identity and uniqueness of each of the 48 chromosome 18-specific transcripts, a homology search against sequence databases was conducted. By comparison using a BLASTN similarity search with GenBank [Altschul, 1990] and a Level I sequence EST homology search of The Institute for Genome Research (TIGR) database [Adams et al., 1995], we found that of the 48 chromosome 18-specific cDNAs, 11 were highly homologous (defined as >89% homology over >100 bp) to segments of five previously known genes (Table III). Myelin basic protein (MBP) [Kamholz et al., 1986], the 63-kDa protein kinase related to ERK3 (HS63KDAP) [Li et al., 1994] and the protein tyrosine phosphatase receptor, mu polypeptide (PTPRM) [Suijkerbuijk et al., 1993] were each represented in four, three, and two clones, respectively. The Gsα, olfactory type (GNAL) [Zigman et al., 1993] and 5′ *H. sapiens* hypothetical protein (HUMKIAAN) [Nomura et al., 1993] were represented in one clone each. In addition, the map assignments obtained for transcripts of these five genes were consistent with previously reported data (Table III and Fig. 2).

A FASTA [Pearson and Lipman, 1988] sequence comparison among the remaining 37 cDNA clones to search for redundancy (defined as ≥89% identical sequence over >100 bp) indicated that 20 cDNAs were unique and 17 redundant cDNAs represented five groups of unique sequences. Therefore, including the transcripts for the known genes, we have identified a total of 30 unique transcripts, of which 25 did not exhibit homology to previously known genes.

TABLE II. Infant Brain Derived cDNA Clones Mapping to Chromosome 18

Clone number	Our insert size (kB)	dbEST insert size (kb)	GenBank accession number 5′	GenBank accession number 3′	Gene homology[a]	EST homology[b]	Cytogenetic bin
1	1.4	1.7	R51685	R51596	HS63KDAP	NA	M
2	1.6	NA	R61592	R61536	Unknown	EST64032	M
3	1.6	2.1	T77500	R38384	HS63KDAP	NA	M
4	1.6	1.6	R56762	R56915	Unknown	Unknown	M
5	1.2	1.4	H08457	H08745	Unknown	Unknown	S
6	1.5	1.5	R54360	R54361	Unknown	Unknown	S
7	1.6	1.9	T78290	R37939	MBP	NA	S
8	1.9	2.4	R20367	R43753	Unknown	Unknown	M
9	1.2	1.2	R18592	R41672	Unknown	EST197262	S
10	1.3	1.4	R18875	R37298	HS63KDAP	NA	M
11	1.5	1.5	R34535	R49065	PTPRM	NA	A
12	1.8	2.0	H17696	H17080	MBP	NA	S
13	1.7	1.9	R52596	R52541	Unknown	Unknown	L
14	1.8	2.0	R13520	R20642	Unknown	Unknown	A
15	1.4	NA	R16321	R41398	Unknown	EST228925	M
16	1.7	NA	H08970	H09539	Unknown	Unknown	S
17	1.6	2.1	R17799	R43004	Unknown	EST64032	M
18	1.5	1.0	R22831	R46021	MBP	NA	S
19	2.0	2.8	R14016	R39139	Unknown	Unknown	M
20	1.1	1.3	R11914	R39106	Unknown	EST197262	S
21	1.5	2.0	R19053	R44040	Unknown	EST228925	M
22	1.1	1.2	R19448	R44696	Unknown	Unknown	C
23	1.1	1.2	T80229	R38716	Unknown	D18S928E	S
24	1.2	1.5	R35001	R49388	Unknown	EST91427	A
25	1.9	2.1	R17655	R43373	Unknown	Unknown	M
26	1.0	1.2	R20441	R44144	Unknown	EST197262	S
27	1.3	1.4	R19332	R44600	Unknown	EST91427	A
28	1.8	NA	H08354	H08355	Unknown	EST91427	A
29	1.8	1.7	None	R39845	Unknown	Unknown	B
30	1.3	1.4	R52394	R52395	Unknown	EST130984	N
31	1.1	1.3	H17749	H17636	GNAL	NA	B
32	1.1	1.1	H06013	H05964	Unknown	EST91427	A
33	1.7	1.9	T74001	T87210	Unknown	Unknown	M
34	1.9	1.3	T80579	R38876	Unknown	Unknown	A
35	1.4	NA	R60481	R60245	Unknown	EST91427	A
36	1.2	NA	R59504	R59505	Unknown	Unknown	K
37	1.9	2.1	R20248	R43704	Unknown	Unknown	B
38	1.1	1.1	H08492	H08770	Unknown	Unknown	M
39	1.6	1.7	H11689	H11600	Unknown	Unknown	G
40	1.7	1.9	R19498	R43846	HUMKIAAN	NA	N
41	1.6	2.0	H17610	H17501	Unknown	Unknown	S
42	1.8	5.9	R17567	R42907	Unknown	EST91427	A
43	1.5	1.5	R20380	R43767	Unknown	Unknown	S
44	1.6	1.6	H17267	H17268	Unknown	EST91427	A
45	1.6	1.6	T80517	R38994	PTPRM	NA	A
46	1.6	1.4	R20075	None	MBP	NA	S
47	1.2	1.3	T66113	T65029	Unknown	Unknown	S
48	1.3	1.3	R15279	None	Unknown	EST91427	A

[a] Determined via BLASTN searches [Altschul, 1990] and intra-group redundancy of >89% over >100 base pairs with another of the 48 clones via FASTA [Pearson and Lipman, 1988].
[b] Determined by searching the UniGene [Boguski and Schuler, 1995] site with the above GenBank account numbers, which showed homology with six UniGene groups (taking into account redundancy), one member of which had been previously mapped to chromosome 18.

The insert sizes of the cDNA clones that were determined to be chromosome 18-specific ranged from 1 to 2 kb (Table II). To explore the presence of an open reading frame (ORF) in each clone and to further examine any homology to known genes, we determined the remaining sequence of the unique clones (data not shown, sequences were deposited in the Genbank with the following accession numbers: U55777 and U55962 to U55991). We found potential polyadenylation signals in some of the clones. So far, no ORFs have been detected, suggesting that a major portion of the cDNA clones corresponded to 3′ UTRs (data not shown). More importantly, comparison of the longer sequences of the cDNAs with sequences in the databases failed to reveal significant homology with any known genes, supporting the idea that these transcripts were derived from novel genes.

Radiation Hybrid Mapping

To achieve a higher resolution map for each of the transcripts by PCR, we used the Stanford G3 radiation hybrid series and primers specific for each cDNA. Of the

TABLE III. Chromosome 18-Specific Brain-Derived cDNAs Homologous to Known Genes

Clone number	BLASTN/TIGR sequence homology	Cytogenetic location	Percentage identical 5′	Percentage identical 3′	Reference
1	63-kDa protein kinase related to ERK3 (HS63KDAP)	18q21.2–18q21.3	98.4	97.5	Li et al. [1994]
3	63-kDa protein kinase related to ERK3 (HS63KDAP)	18q21.2–18q21.3	95.3	96.2	Li et al. [1994]
7	Myelin basic protein (MBP)	18q23	95.8	93.5	Kamholz et al. [1986]
10	63-kDa protein kinase related to ERK3 (HS63KDAP)	18q21.2–18q21.3	99.6	98.4	Li et al. [1994]
11	Protein tyrosine phosphatase, receptor-type, mu polypeptide (PTPRM)	18p11.2	None	89.2	Suijkerbuijk et al. [1993]
12	Myelin basic protein (MBP)	18q23	95.7	92.1	Kamholz et al. [1986]
18	Myelin basic protein (MBP)	18q23	94.5	98.1	Kamholz et al. [1986]
31	Guanine nucleotide-binding protein, α-subunit, olfactory type (GNAL)	18p11.22–p11.21	98.9	98.6	Zigman et al. [1993]
40	5′ *H. sapiens* hypothetical protein (HUMKIAAN)	18q21.3–18qter	98.4	None	Nomura et al. [1995]
45	Protein tyrosine phosphatase, receptor-type, mu polypeptide (PTPRM)	18p11.2	94.0	90.0	Suijkerbuijk et al. [1993]
46	Myelin basic protein (MBP)	18q23	95.3	None	Kamholz et al. [1986]

25 unique transcripts, 19 were successfully linked to chromosome 18 STSs (Table IV and Fig. 3). The positions of the cDNAs in the radiation hybrid framework map were consistent with their subchromosomal assignments (Figs. 2 and 3). With this method, fine mapping was established for the unique transcripts as evidenced by the physical distance between them and the chromosome 18 STSs, which ranged from ≈4 to 46 cR, estimated to be between ≈100 and 1100 kb (Figs. 2 and 3).

Radiation hybrid mapping was also used to position the known genes identified in this study against the 25 nonredundant transcripts. We found that HS63KDAP formed a high resolution linkage group with clones 2, 4, 19, and 33 (Table IV and Fig. 3). The fine map locations of the anonymous markers D18S37, D18S53, and D18S40 were similarly examined, since they showed excess allele sharing in bipolar affected sib-pairs in two studies [Berrettini et al., 1994; Stine et al., 1995]. These three markers, GNAL and cDNA clones 22, 24, and 37, assembled into a separate radiation hybrid linkage group (Table IV and Fig. 3). Further investigation into the linkage overlaps between the STSs, genes, and unique transcripts showed that at least six radiation hybrid linkage groups were evident (Fig. 3). Based on these physical relationships, a map order within each linkage group could be deduced.

Six unique transcripts and two of the known genes (PTPRM and HUMKIAAN) detected in this study could not be mapped with this radiation hybrid series raising the possibility that a higher resolution radiation hybrid panel may be required (Table IV).

DISCUSSION

Using direct cDNA selection and physical mapping by PCR, we have identified and positionally catalogued 48 chromosome 18-specific cDNAs that are expressed in infant brain. Sequence database comparison revealed a level of redundancy in the 48 clones, yielding a total of 30 unique transcripts. Five genes previously assigned to chromosome 18 were represented in these transcripts. Additional sequence analysis of the remaining 25 nonredundant cDNA clones and database comparisons failed to elicit any significant homology to known genes, indicating that these brain-expressed transcripts represent novel genes.

So far, we have no evidence for possible redundancies among the unique transcripts due to alternative splicing or the presence of pseudogenes, but these probably are very minor components of the cDNA library. Polymeropoulos et al. [1993] suggested the possibility that chromosome 18 may be gene-poor. A recent effort to sequence and map cDNAs yielded only four on chromosome 18 out of the several hundred cDNAs localized to other chromosomes [Berry et al., 1995]. The 25 unique cDNA clones isolated in this study, therefore, represent a significant increase in the number of new genes on chromosome 18.

High resolution mapping with the use of a radiation hybrid panel reveals that the brain transcripts detected through cDNA selection assemble into six linkage groups. Surprisingly, in this initial analysis, the majority of the cDNAs appear to cluster to three main regions

TABLE IV. Radiation Hybrid Mapping of Unique cDNA Clones*

Test marker	Reference marker	LOD	Distance to reference marker ($_cR_{8000}$)
14	D18S476	10.2	25.78
	D18S481	13.3	15.04
	D18S54	8.9	32.31
	D18S63	10.9	22.50
	D18S459	10.2	25.78
	D18S1132	7.0	42.16
34	D18S476	8.3	32.61
	D18S481	12.4	16.08
	D18S54	12.7	15.96
	D18S63	14.6	9.61
	D18S459	14.6	9.61
	14	7.6	38.31
	D18S1132	6.8	41.79
24	D18S464	11.0	16.07
	D18S53	6.1	45.87
GNAL	D18S482	7.8	36.15
	D18S71	9.0	31.62
D18S53	D18S464	7.5	35.77
	D18S482	7.0	39.77
	D18S71	6.9	42.39
37	D18S73	7.1	40.23
	D18S71	8.0	35.03
22	D18S73	7.7	37.31
	D18S40	13.3	16.92
D18S40	D18S73	11.2	21.40
	D18S71	7.3	40.07
D18S37	D18S73	13.5	16.28
	D18S71	7.6	39.76
39	D18S1101	7.8	37.31
13	D18S1160	7.8	28.72
	D18S475	7.7	19.22
25	D18S454	10.3	10.73
8	D18S460	7.8	28.65
	D18S72	7.8	28.72
15	D18S460	7.2	30.83
	D18S72	8.5	24.10
	8	12.5	8.60
4	D18S470	6.1	42.49
	19	6.6	35.80
2	**19**	14.3	4.08
	D18S470	12.6	11.47
	4	6.0	40.67
	D18S474	8.3	24.21
	D18S69	8.2	27.02
HS63KDAP	**2**	10.5	17.02
	D18S474	9.8	18.18
	19	9.1	22.46
	D18S470	7.7	33.44
	D18S69	6.5	38.01
19	D18S470	10.3	19.96
	D18S474	8.4	24.51
	D18S69	7.1	33.41
33	D18S69	7.1	33.41
41	D18S486	6.5	33.52
	D18S58	7.2	30.83
	23	15.1	3.85
23	D18S58	6.6	35.71
43	**23**	14.4	7.28
	41	12.7	11.44
	D18S58	6.5	35.88
MBP	D18S554	7.8	25.97
5	D18S70	7.0	21.37
6	D18S70	6.3	28.01
9	NL		
16	NL		
29	NL		
30	NL		
36	NL		
47	NL		
HUMKIAAN	NL		
PTPRM	NL		

* A description of the Stanford G3 radiation hybrid panel is in the text. Markers in bold represent cDNAs identified in this study or anonymous STSs. NL, markers or transcripts which could not be mapped to the radiation hybrid framework markers.

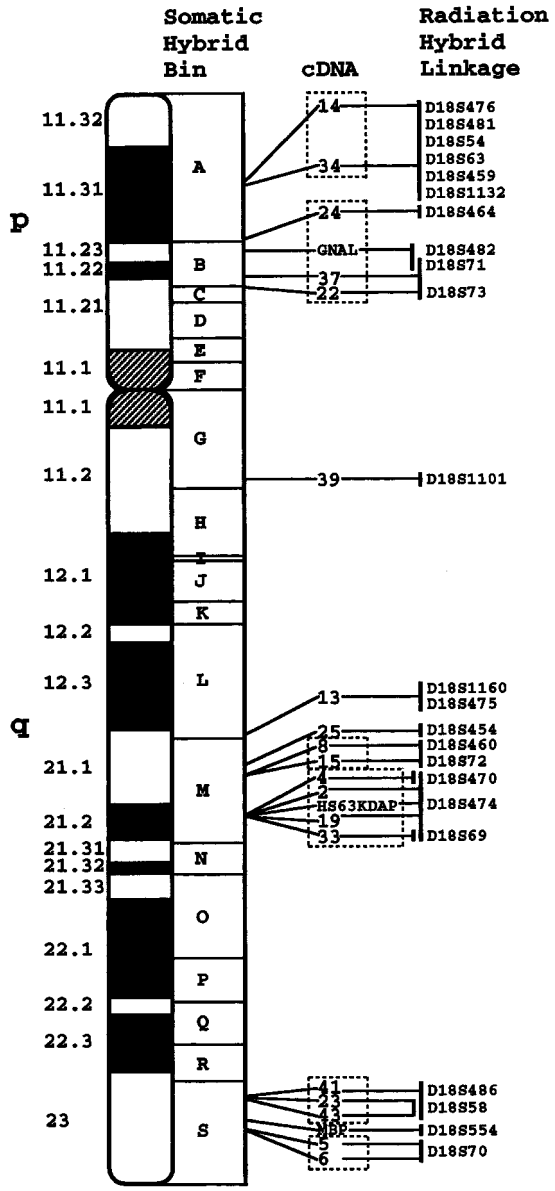

Fig. 3. High-resolution mapping of transcripts versus chromosome 18 reference STS by the use of radiation hybrids. A schematic representation of the position of the unique transcripts with respect to linked STSs. Transcripts and genes that are members of a radiation hybrid linkage group are enclosed in dashed boxes. The approximate locations within the cytogenetic bins are also indicated.

of the chromosome, one on 18p and two on 18q. This clustering of transcripts might reflect gene-rich regions, although this may also simply be a stochastic phenomenon. Alternatively, a bias could have occurred in the cloning process or in the uneven growth of individual cosmids in each pool.

Interestingly, the "transcript-rich" sites found in this study coincide with those proposed as susceptibility regions for bipolar disorder. These regions are as follows: at around 18p11.2–p11.3 [Berrettini et al., 1994; Stine et al., 1995], 18q21.2–q21.3 [Stine et al., 1995], and 18q22–q23 [Freimer et al., 1996]. (Cytogenetic locations were taken from Overhauser et al. [1995].) The high-resolution map creates a radiation hybrid linkage group composed of three transcripts and GNAL, and the anonymous markers D18S37, D18S53, and D18S40 on 18p (Fig. 3), loci in which excess allele sharing in affected sib-pairs of bipolars has been demonstrated [Berrettini et al., 1994; Stine et al., 1995]. Seven transcripts and HS63KDAP assemble into two radiation hybrid linkage groups in the M bin of 18q, previously suggested to contain a susceptibility locus, with a parent-of-origin effect [Stine et al., 1995]. Two of the regions that contain a cluster of the brain transcripts overlap with breakpoints for inversion on chromosome 18, inv(18) (p11.3; q21.1), observed in bipolar patients [Mors et al., 1995]. We have also assigned another nine new transcripts, five of which form two additional radiation hybrid linkage groups, to the region of linkage suggested by Freimer et al. [1996]. In conclusion, we have isolated, sequenced, and mapped brain-expressed transcripts, contained in yet undescribed genes, which may be positional candidates for bipolar disorder, and mapped them to within ≅100 to 1100 kb of reference markers to aid in the hunt for the bipolar disorder susceptibility loci.

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